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Una nueva especie de *Telmatobius* (Anura, Leptodactylidae) de la ceja de montaña de La Paz (Bolivia)

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A new species of *Telmatobius* is reported from the humid regions of the "ceja de montaña" of Kkota Pata, Department of La Paz, Bolivia. This species is characterized by its large eyes and the structure of the skin, with wide and protruded semicircular warts.

INTRODUCCIÓN

En la batracofauna de Bolivia se han registrado hasta el momento tres especies de *Telmatobius* que son habitantes estrictos de cursos de agua en bosques de montañas: *Telmatobius bolivianus* Parker, 1940, *T. verrucosus* Werner, 1899 y *T. yuracare* De La Riva, 1994. Por otra parte, *T. simonsi* Parker, 1940, descripta originalmente para Sucre, en áreas de valles mesotérmicos, fue citada por DE LA RIVA (1990, 1994) en regiones boscosas de La Siberia, en el Departamento Cochabamba, aunque esta identificación es dudosa.

Entre las formas estrictamente silvícolas, el estado taxonómico de *T. verrucosus* es confuso. VELLARD (1951) consideró a *T. bolivianus* como un sinónimo más reciente de esta especie, y posteriormente (VELLARD, 1970), consideró a ambas como subespecies de *Telmatobius marmoratus*. En un trabajo reciente (LAVILLA & DE LA RIVA, 1993), basados en caracteres larvales, se determinó que *Telmatobius bolivianus* es una buena especie, y la posición de *T. verrucosus* es todavía un interrogante abierto.

En un estudio sobre los anfibios de la región de Kkota Pata, en el Departamento La Paz, encontramos una población de *Telmatobius* en un arroyo de la ceja de montaña, con

caracteres notablemente diferentes a los de los restantes *Telmatobius* conocidos, y en especial de aquellos que habitan los cuerpos de agua de los bosques de montaña en Bolivia, lo que nos lleva a describirla como nueva.

Finalmente, conviene destacar que las selvas de montaña del oeste de América de Sur parecen albergar un interesante conjunto de novedades dentro del género *Telmatobius*, tal como se desprende de la síntesis presentada por Laurent (1980) para Argentina y de los trabajos de De La Riva (1990, 1994) para Bolivia y Wiens (1993) para el norte de Perú.

Métodos

La descripción que se presenta a continuación ha considerado los caracteres clásicos de morfología externa. El material estudiado fue fijado en formol 10 % en cámara húmeda durante 24 horas y conservado en alcohol etílico de 70°. Un ejemplar macho adulto fue teñido y diafanizado siguiendo la técnica propuesta por Wassersug (1976); aunque en el presente trabajo no se presenta una descripción del esqueleto, un carácter osteológico, la estructura del húmero, ha sido empleado para diferenciar la nueva especie de *Telmatobius yuracare*.

Las medidas fueron tomadas bajo lupa binocular, empleando un calibre con precisión de 0,02 mm.

Resultados

Telmatobius jahuira sp. nov.
(fig. 1)

Holotipo. — Colección Boliviana de Fauna (CBF) 01675, macho adulto, colectado por E. Lavilla y P. Ergueta el 21 de abril de 1992 en el Río Chairo, en las proximidades de la Mina Copacabana (aproximadamente 16°16'S 67°50'W), Kkota Pata, Departamento La Paz, Bolivia.

Paratipos. — Alotipo: CBF 01676, hembra adulta, mismos datos que el holotipo. Otro paratipo: CBF 01571, macho adulto, colectado por R. Hinojosa y S. Otazu el 21 de abril 1992 en Kkota Pata, Departamento La Paz, Bolivia.

Etimología del nombre específico. — Jahuira es un vocablo aymara que significa río, y se lo emplea en alusión a los hábitos de esta especie. Aquí es empleado como sustantivo.

Diagnosis. — *Telmatobius jahuira* está caracterizada por el notable desarrollo de sus ojos, grandes y sobresalientes, y la piel dorsal con verrugas grandes, hemisféricas, separadas entre sí y pigmentadas de negro, lo que hace que la librea aparezca con numerosas manchas aproximadamente circulares. Las diferencias con los otros *Telmatobius* que son habitantes exclusivos de ambientes selváticos están puntualizadas en la discusión.

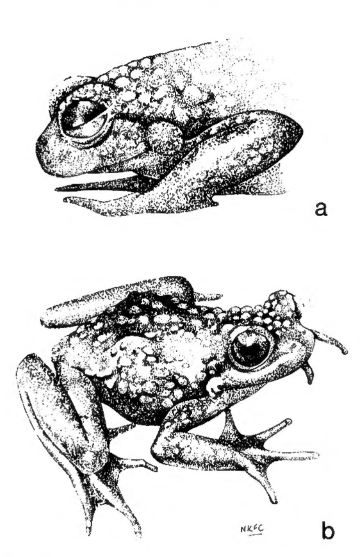


Fig. 1. — (a) Detalle de la cabeza en vista lateral de *Telmatobius jahuira*. (b) Vista general del holotipo de *Telmatobius jahuira* (longitud hocico-cloaca: 55,6 mm).

Descripción del holotipo. — Longitud hocico-ano: 55,6 mm. La cabeza es deprimida, con la región gular plana; es más ancha (21,3 mm) que larga (18,1 mm), con indice cefálico de 0,85.

El hocico es redondeado en vistas dorsal y lateral, y su longitud es menor (4,6 mm) que el diámetro del ojo (6,5 mm). El canthus rostralis, marcado, es redondeado y la región loreal es cóncava. Los labios están levemente engrosados, no son glandulares y se proyectan por sobre la mandíbula inferior en todo su perímetro.

Los ojos son proporcionalmente grandes (corresponden a aproximadamente el 36 % de la longitud de la cabeza) y son muy sobresalientes. La comisura posterior de los párpados está más lejos de la boca (6,2 mm) que el margen ventral de los orificios nasales (4,3 mm). La pupila es redonda y la membrana nictitante es transparente, con una banda pigmentada, en la que alternan secciones grises y amarillas, en el margen libre. El diámetro del ojo es mayor que la distancia del ojo a la narina (3,7 mm), y la distancia interocular anterior (tomada entre las comisuras anteriores de los párpados) equivale al 47 % del ancho de la cabeza. Los ojos tienen posición más lateral que frontal.

Desde la región posterior del ojo se extiende, hacia atrás y hacia abajo, un pliegue supratimpánico engrosado y glandular, que termina a la altura de la implantación del miembro anterior. Por debajo de este pliegue y por detrás de la boca se ubica una glándula postcomisural muy marcada, redondeada, elevada, rugosa y sin cornificaciones.

El tímpano y el anillo timpánico no están diferenciados externamente y la región timpánica es glandular. Los orificios nasales son subcirculares, rebordeados y no están elevados. La distancia internasal (3,5 mm) es levemente menor que la distancia naso-ocular (3,7 mm), y 2,8 veces menor que la distancia interocular anterior. La lengua es redonda, entera y libre posteriormente.

La región dorsal del cuerpo y la cabeza presentan numerosas glándulas subcirculares, proporcionalmente grandes, elevadas y sin cornificaciones, que están separadas entre sí por áreas rugosas. La región dorsal de los miembros anteriores y posteriores es rugosa y carece de acúmulos glandulares elevados. La región ventral es uniformemente lisa, y no existen cornificaciones nupciales en el pecho.

El orificio cloacal es posterodorsal (ubicado a la altura de la región media de los muslos) y no existe un pliegue cloacal definido; en su lugar, toda la región pericloacal aparece estrechamente plisada. El pliegue supracloacal es breve y recto.

Los dedos de las manos poseen falanges terminales redondeadas y levemente expandidas. El tubérculo metacarpal interno es oval, estrecho, protuberante y 1,4 veces más largo que el externo, suboval y ancho. Entre ambos tubérculos metacarpales existe un tubérculo grande, que puede ser interpretado como un tercer tubérculo metacarpal, como un tubérculo palmar hipertrofiado o como un tubérculo supernumerario del dedo I. Los tubérculos subarticulares son redondeados, sobresalientes y se disponen según la fórmula I (1), II (1), III (2), IV (2). La palma de la mano es lisa, con escasos tubérculos redondeados y pequeños. La palmeadura está reducida a un reborde cutáneo en el margen externo del pollex. La longitud relativa de los dedos es III > IV > I > II.

Los dedos de los pies llevan falanges terminales redondeadas y no dilatadas. El tubérculo metatarsal interno es oblongo y mayor que el externo, que es oval. No existen tubérculos plantares, y los tubérculos subarticulares, que en general son ovales, sobresalientes y enteros, se disponen según la fórmula I (1), II (1), III (2), IV (3), V (2). La palmeadura está mejor desarrollada entre los dedos II-III y III-IV, y alcanza su menor desarrollo entre los dedos I-II; en todos los casos, alcanza el extremo de los dedos por medio de rebordes cutáneos. El pliegue tarsal está medianamente desarrollado en el margen interno del hallux, y por detrás del tubérculo metatarsal interno se continúa como una línea clara. En el margen externo del dedo IV existe un pliegue cutáneo que se extiende

desde el tubérculo subarticular basal hasta el extremo del dedo. Cuando los fémures son colocados en ángulo recto con respecto al eje axial del cuerpo, los talones se superponen y cuando la pata es llevada hacia adelante, la articulación tibio-tarsal alcanza la mitad del ojo. La relación entre las diversas regiones del miembro posterior y la longitud total del cuerpo son: fémur, 53,2 %; tibia-fíbula, 54,1 %; tarso, 27,5 %; pie, 58,4 %.

Los caracteres sexuales secundarios se restringen a las callosidades nupciales que se ubican en el margen interno y la cara dorsal del pollex, y se presentan como escasas espinas córneas que dejan amplios espacios no queratinizados entre sí.

Coloración en vida: dorso de cabeza, cuerpo y miembros verde oliva; manchas aproximadamente circulares negras, de límites netos, en el dorso del cuerpo y la cabeza; región dorsal de los miembros con manchas irregulares; ventralmente grisáceos, con manchas amarillo-naranja, más notorias en los miembros anteriores y posteriores y sobre el pecho.

Coloración en fijador: dorsalmente gris oscuro con manchas aproximadamente circulares negras; ventralmente, gris mediano, con manchas beige.

Notas sobre los paratipos. — Se señalan sólo aquellos caracteres morfológicos que divergen del holotipo, y las medidas correspondientes son presentadas más abajo.

Alotipo CBF 01676. — El perfil de la región gular es redondeado. Las manchas negras del dorso del cuerpo y de la cabeza son más numerosas, mayores y algunas pueden presentar contornos irregulares, por coalescencia de manchas próximas. No existen rebordes cutáneos en el pollex, y los tubérculos palmares son más numerosos y están mejor definidos que en el holotipo; existe un tubérculo supernumerario en el dedo I (derecha); no existen callosidades nupciales. En la pata existe un tubérculo plantar entre los tubérculos metatarsales.

Otro paratipo CBF 01571. — Con excepción de los caracteres morfométricos (ver más abajo), no existen diferencias morfológicas de importancia con el holotipo.

Medidas. — Las medidas están expresadas en milímetros; el primer valor corresponde al holotipo, el segundo al alotipo y el tercero al otro paratipo. Longitud del cuerpo: 55.6; 58.9; 55.1. Longitud de la cabeza: 18.1; 18.8; 17.1. Ancho de la cabeza: 21.3; 21.2; 19.8. Longitud del hocico: 4.6; 4.6; 4.1. Distancia naso-ocular: 3.7; 3.9; 3.8. Distancia internasal: 3.5; 3.9; 3.7. Distancia interocular anterior: 10.0; 9.2; 9.2. Distancia interocular posterior: 17.9; 18.1; 17.3. Tubérculo metacarpal interno: 5.3; 4.5; 5.6. Tubérculo metacarpal externo: 3.8; 3.8; 4.2. Diámetro del ojo: 6.5; 6.8; 6.8. Diámetro del orificio nasal: 0.68; 0.70; 0.56. Distancia ojo-boca: 6.2; 5.9; 5.7. Distancia nariz-boca: 4.3; 4.6; 3.9. Longitud del fémur: 29.6; 31.6; 28.9. Longitud de la tibia: 30.1; 32.0; 30.1. Longitud del tarso: 15.3; 15.4; 14.8. Longitud del pie: 32.5; 34.2; 32.2.

Habitat. — La localidad tipo se encuentra en la región de ceja de montaña del Departamento La Paz, y el clima regional se caracteriza por presentar un régimen húmedo a perhúmedo. Los datos obtenidos de la estación meteorológica de Chururaqui, en el vecino valle de Zongo, muestran precipitaciones de 3250 mm anuales.

Holotipo y alotipo fueron coleccionados en las primeras horas de la tarde; estaban inactivos, en el agua y bajo piedras planas, grandes. El esfuerzo de búsqueda indicaría que se trata de una especie poco abundante en la localidad tipo.

Discusión

Las cuatro especies de *Telmatobius* reportadas para las selvas de montaña de Bolivia son, como señaláramos en la introducción, *Telmatobius bolivianus*, *T. verrucosus*, *T. yuracare* y la nueva especie que describimos aquí, *T. jahuira*.

Telmatobius jahuira se diferencia de *T. verrucosus* (según las descripciones disponibles de Werner, 1899 y Vellard, 1951) por presentar: (1) el hocico más corto que el diámetro del ojo; (2) el canthus rostralis más marcado; (3) las narinas más cerca del ojo que del hocico; (4) el primer dedo de la mano más largo que el segundo; (5) la membrana interdigital poco desarrollada entre los dedos II-III y III-IV de la pata; (6) el pliegue tarsal vestigial; (7) la mandíbula superior proyectada sobre la inferior en todo su perímetro; (8) un patrón de coloración dorsal con color de base verde oliva y manchas circulares negras.

Telmatobius jahuira difiere de *T. bolivianus* (según la descripciones de Parker, 1940 y Vellard, 1951) por presentar: (1) el hocico redondeado y más corto que el diámetro ocular; (2) los dedos de las manos con falanges terminales redondeadas, levemente expandidas; (3) el primer dedo de la mano más largo que el segundo; (4) los dedos II y III de las manos sin reborde cutáneos; (5) los extremos de los dedos de las patas no dilatados; (6) la membrana interdigital poco desarrollada entre los dedos II-III y III-IV; (7) el pliegue tarsal vestigial; (8) la articulación tibio-tarsal alcanzando a la mitad del ojo; (9) la piel con glándulas muy desarrolladas; (10) un patrón de coloración diferente; (11) la estructura, abundancia y disposición de las espinas córneas en los pulgares de los machos.

Telmatobius jahuira difiere de *T. yuracare* (según la descripción de De La Riva, 1994), entre otros caracteres, por: (1) carecer de espina humeral en los machos; (2) la orientación de los ojos; (3) la estructura de la piel del dorso del cuerpo; (4) la textura de la piel del dorso del cuerpo; (5) el patrón de coloración.

Cualquiera sea la posición taxonómica definitiva de los taxa yungueños del género *Telmatobius* en Bolivia, se observa claramente que *T. jahuira* es una especie diferente de las previamente descriptas.

Desafortunadamente no contamos aún con larvas de esta especie, por lo que no podemos atribuirla a ninguno de los dos grupos conocidos (Lavilla, 1985).

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Reassessment of central Peruvian Telmatobiinae (genera *Batrachophrynus* and *Telmatobius*). II. Allozymes and phylogenetic relationships

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Three hypotheses on the phylogenetic relationships among central Peruvian Telmatobiinae were tested: (1) the common ancestor of the two *Batrachophrynus* species diverged from the telmatobiine stock independently from the common ancestor of the present *Telmatobius*; (2) *B. macrostomus* and *B. brachydactylus* separated independently from the lineage leading to the present *Telmatobius*; (3) the separation of the common *Batrachophrynus* ancestor from *Telmatobius* occurred after the differentiation of the *Telmatobius* stock into geographical lineages. Allozymes clearly indicate the monophyly of *Batrachophrynus*, and that the southern Peruvian *T. culeus* is more closely related to the central Peruvian *T. jelskii* and *T. rimac* than to either of the *Batrachophrynus* species. All available evidence supports the first hypothesis.

INTRODUCTION

Three genera of central Peruvian Telmatobiinae are currently recognized: *Telmatobius* Wiegmann, 1835, *Batrachophrynus* Peters, 1873 and *Lynchophrys* Laurent, 1983 (FROST, 1985; DUELLMAN, 1993). A taxonomic reassessment of the six species assigned to these genera indicated that the monotypic genus *Lynchophrys* is not valid and that *Lynchophrys brachydactyla* should be referred to as *Batrachophrynus brachydactylus* (SINSCH et al., 1995). In contrast, osteological evidence (LYNCH, 1978) and morphometric data (SINSCH et al., 1995) continue to support the validity of the genera *Batrachophrynus* and *Telmatobius*. The corresponding phylogenetic hypothesis assumes a monophyletic origin of both genera (CEI, 1986).

LAURENT (1983), however, proposed an alternative hypothesis. He assumed an early separation of *B. macrostomus*, and an independent, but later, separation of *B. brachydactylus* from the *Telmatobius* stock. If this was true, the morphometric and osteological similarities between the two *Batrachophrynus* species would be convergences and the genus would be paraphyletic. There is a third possibility, considering the conspicuous general similarity between the two genera: the separation of the *Batrachophrynus* ancestor from the

central Peruvian *Telmatobius* stock may have occurred after the splitting of *Telmatobius* into a northern and a southern lineage. In this case, the genus *Batrachophrynus* would be invalid and its two species would have to be included in the genus *Telmatobius*.

The aim of our study was to test these alternative hypotheses by analyzing the genetic similarity among four central Peruvian and a southern Peruvian species of Telmatobiinae. The stream-dwelling *T. jelskii* (Peters, 1873), *T. rimac* Schmidt, 1954 and *B. brachydactylus* Peters, 1873, and the lake-dwelling *B. macrostomus* Peters, 1873, inhabit neighbouring regions within the Departments of Cerro de Pasco, Junín and Lima. The Titicaca frog *T. culeus* (Garman, 1875), which represents another evolutionary lineage within the genus *Telmatobius* (VELLARD, 1951, 1953, 1955), was included as a potential outgroup. Horizontal starch gel electrophoresis of the proteins of blood, muscle and liver homogenates was used to assess allelic variation in homologous loci among all populations and species. Our reconstruction of phylogenetic relationships within this Andean group of Telmatobiinae clearly supports CEI's (1986) hypothesis of a monophyletic origin of *Batrachophrynus* and *Telmatobius*.

MATERIAL AND METHODS

We examined 111 frogs representing five species of Telmatobiinae (Leptodactylidae): (1) *Batrachophrynus brachydactylus*: 11 males and 11 females from a brook near Ondores (Dep. Junín, Perú); (2) *B. macrostomus*: 6 males, 7 females and 8 juveniles from Junín Lake (Dep. Junín); (3) *Telmatobius jelskii*: (a) 5 males, 2 females and 14 juveniles from Rio Shullcas, Palian near Huancayo (Dep. Junín), (b) 6 males and 3 females from Cuyrohuasi near Tarma (Dep. Junín); (4) *T. rimac*: (a) 7 males, 7 females and 8 juveniles from Rio Chillón, Obrojillo near Canta (Dep. Lima, Perú), (b) 9 males, 4 females and 2 juveniles from Quebrada Huaytara, Canta (Dep. Lima); (5) *T. culeus*: 1 male from Titicaca Lake (Dep. Puno, Perú).

Following the morphological classification of specimens (SINSCH, 1986, 1990; SINSCH et al., 1995), we collected blood samples (about 60 µl per individual) from the vena angularis of the living frogs (NÖLLER, 1959). Samples were centrifuged at 11,500 rpm for 3.33 min and the cell fraction was dissolved in 20 µl homogenate buffer (tris-EDTA-NADP at pH 7.0) and stored at -18°C until use. The frogs were sacrificed and liver and muscle samples were taken and mechanically homogenated in 50-150 µl in tris-EDTA-NADP buffer at pH 7.0. All samples were collected in Perú in 1992 except for the blood sample of the *T. culeus* specimen. This individual was kept in the Museum of Natural History in Bonn, Germany, and was included to look for fixed alleles in the *Telmatobius* and *Batrachophrynus* lineages.

Gels for horizontal electrophoresis were prepared at 12 % with SIGMA starch. Four buffer systems were used at constant 55 mA and 4°C: (1) tris-citrate (electrode: pH 8.0; gel: pH 8.7), duration of electrophoresis: 5 h; (2) tris-malate (electrode and gel: pH 8.4), 4 h; (3) tris (electrode and gel: pH 8.6), 5 h; (4) tris-borate (electrode and gel: pH 9), 4 h. Each

gel was sliced into five 2 mm thick slabs for staining. Procedures for staining were those described by Pasteur et al. (1988), Shaw & Prasad (1970) and Sherif (1990).

Allozymes examined were representative of 12 enzyme systems controlled by a total of 22 presumptive loci: aspartateamino transferase (1 locus, abbreviation: AAT, E.C. No. 2.6.1.1); adenylate kinase (1, AK, 2.7.4.3); esterase (4, EST, 3.1.1.1); glucosephosphate isomerase (2, GPI, 5.3.1.9); hexanol dehydrogenase (2, HDH, 1.1.1.56); isocitrate dehydrogenase (2, IDH, 1.1.1.42); lactate dehydrogenase (2, LDH, 1.1.1.27); malate dehydrogenase (2, MDH, 1.1.1.37); malic enzyme (1, ME, 1.1.1.40); peptidase (3, PEP, 3.4.1.1); 6-phosphogluconate dehydrogenase (1, 6-PGD, 1.1.1.44); phosphoglucomutase (2, PGM, 2.7.5.1). In addition, we scored the non-enzymatic hemoglobin (Hb).

Multiple loci were numbered from cathode to anode. Presumptive alleles were designated numerically according to their mobility relative to the most common electromorph (assigned 100) of the *T. jelskii* population from Palian. Faster moving electromorphs were assigned higher values (above 100), slower moving ones lower values (below 100). For reference, each electrophoretic run included samples of the *T. jelskii* population from Palian. Statistical analyses included the calculation of allele frequencies, of Nei's genetic distance (Nei, 1972), Cavalli-Sforza's chord distance (Cavalli-Sforza & Edwards, 1967) and Reynolds's genetic distance (Reynolds et al., 1983) by the program GENDIST 3.4 (package PHYLIP; Felsenstein, 1985). Average heterozygosity per locus (Ho = observed frequency; He = expected frequency), proportion of polymorphic loci (P %), and the mean number of alleles per locus (A) were calculated for each sample except *T. culeus*. We used the G-test to detect deviations of observed heterozygosity from the Hardy-Weinberg equilibrium.

Reconstruction of phylogenetic relationships between the examined populations is based on four algorithms applied to allele frequencies: (1) UPGMA method (program NEIGHBOR 3.41); (2) Fitch-Margoliash method assuming equal rates of evolutionary change in all lineages (KITSCH 3.41); (3) Fitch-Margoliash method without evolutionary clock (FITCH 3.41); (4) maximum likelihood method (CONTML 4.42). All calculations are based on the cited programs in the package PHYLIP (Felsenstein, 1985).

Results

Allelic variations of proteins

A total of 23 presumptive loci (enzymes: AAT, AK, EST, GPI, HDH, IDH, LDH, MDH, ME, PEP, 6PGD, PGM; non-enzymatic protein: Hb) was scored in 6 populations of four central Peruvian telmatobiine species and in one specimen of *T. culeus* (Table I). Five loci were monomorphic in all samples: HDH1, LDH2, PEP2.1, 6PGD and PGM1. Allele frequencies are listed in Table I. The observed heterozygosity significantly deviated from the expected heterozygosity of the Hardy-Weinberg equilibrium in all populations because of a deficit of heterozygotes at all loci (G-test, $P < 0.001$). The following account of the loci demonstrates that fixed alleles at the LDH1 locus permit an unequivocal

distinction between the genera *Batrachophrynus* and *Telmatobius*, and that the southern Peruvian *T. culeus* differs from all central Peruvian populations by the presence of an unusual allele at the MDH1 locus.

Aspartateamino transaminase

Following electrophoresis in the tris-malate system, we regularly detected activity in muscle and liver samples. The *Batrachophrynus* species are monomorphic for allele 100, whereas the *Telmatobius* populations possess a second, more slowly migrating AAT (allele 60). Heterozygotes were not observed.

Adenylate kinase

All blood and liver samples show stainable activity following electrophoresis in the tris-borate system. The frequencies of the main alleles 100 and 120 are similar in all samples. One specimen of *B. brachydactylus* possessed a third allele 75. Heterozygotes were not observed.

Esterase

We detected four loci which can easily be distinguished by their specific activity in different tissues and their electrophoretic mobility in the tris system. The most slowly moving esterase (EST1) usually shows low activity in the liver samples and sometimes also in the blood samples, whereas EST2 is active in all samples but stains most strongly in the liver samples. The faster moving esterases (EST3, EST4) produce stainable bands with about the same activity in all samples. The frequencies of the main alleles 90 and 100 of the EST1 locus are similar in all populations. Two *B. brachydactylus* showed a third allele. Two alleles 90 and 100 of EST2 locus are present in both *Batrachophrynus* species and in *T. rimac*, whereas in *T. jelskii* this locus is monomorphic. With the exception of two heterozygotes of the constitution 50/100 (*B. brachydactylus*) at the EST1 locus, all other specimens are homozygotes for the four esterase loci.

Glucosephosphate isomerase

In the tris-borate system an anodally migrating GPI was active in blood and liver samples. Allele 100 dominates in all populations, except for those of *T. rimac* which are almost monomorphic for allele 73. No heterozygotes were detected.

Hexanol dehydrogenase

The tris-malate system resolved two systems of HDH, the monomorphic HDH1 locus being active at similar levels in all tissues and the polymorphic HDH2 locus with stainable

activity in muscle and liver samples. The frequency of allele 100 is greater in the *Batrachophrynus* species than in the *Telmatobius* species where allele 75 dominates. A single heterozygote of the constitution 75/100 (*B. macrostomus*) was detected.

Isocitrate dehydrogenase

Two polymorphic loci coding for enzymes of considerably different electrophoretic mobility were detected in the tris-citrate system. Both loci were almost exclusively active in muscle and liver samples. In *Telmatobius* the IDH1 locus is monomorphic for the allele 100, whereas in *Batrachophrynus* a second, rare allele 93 is present. Four alleles are found at the IDH2 locus with allele 100 dominating in *Telmatobius*. In *Batrachophrynus* allele 90 is the most common one. A single heterozygote of the constitution 100/125 (*B. brachydactylus*) was detected.

Lactate dehydrogenase

Following electrophoresis in the tris-citrate system, only one band stained in the muscle and liver samples of all species, whereas in the blood samples up to five bands appeared. The common stainable band of all tissues is the tetramer of the unit coded for by the LDH1 locus. The five banded pattern was detected only in the blood of the two *Batrachophrynus* species, whereas in the blood of the *Telmatobius* species at most three bands stained, corresponding to slowly moving tetramers. The LDH1 locus is diagnostic for the distinction of the two genera: all *Telmatobius* are fixed for the allele 100, all *Batrachophrynus* for the allele 33. Pure tetramers of the unit coded for by the LDH2 locus were found in both *Batrachophrynus* with the same electrophoretic mobility (allele 100). The poor resolution of the one or two bands of mixed tetramers in *Telmatobius* does not permit a reliable estimate of the position of the non-expressed pure LDH2-tetramer.

Malate dehydrogenase

The best resolution of the bands corresponding to two polymorphic MDH loci was found in the tris-borate system. The MDH1 locus produces a slowly moving protein which was exclusively active in blood and liver samples. In contrast, the faster moving product of the MDH2 locus was present in all tissues, but, especially in the muscle samples, subbands frequently appeared which were not present in the other tissues of the same individual. The MDH1 locus is diagnostic for the southern Andean *T. culeus* which possesses allele 125, whereas only alleles 71 and 100 are present in all central Peruvian populations. The frequencies of allele 71 are extremely low in both *Batrachophrynus*, but considerably greater in *Telmatobius*. Three alleles are found at the MDH2 locus, the usually dominating allele 100, the less frequent allele 85 and the rare allele 115. Only one heterozygote of the constitution 85/100 (*T. rimac*, Obrojillo) was detected at the MDH2 locus.

Malic enzyme

The polymorphic ME locus accounted for regular activity in all samples following electrophoresis in the tris-malate system. Allele 100 dominates in all populations and reaches almost monomorphic frequencies in *T. jelskii*. *T. rimac* and the two *Batrachophrynus* differ from this species by a considerably higher frequency of allele 60 and the presence of the rare allele 20. At this locus we detected 12 heterozygotes: six of the constitution 20/60 (3 *B. brachydactylus*, 1 *B. macrostomus*, 2 *T. rimac* from Obrojillo), and another six of the constitution 60/100 (1 *B. brachydactylus*, 1 *B. macrostomus*, 3 *T. rimac* from Obrojillo, 1 *T. rimac* from Huaytara).

Peptidase

Following electrophoresis in the tris-citrate system, we identified three PEP loci which were monomorphic in most populations. The peptidase of the PEP1 locus specifically digested the dipeptide VAL-LEU and had greater activity in blood and liver samples than in muscle tissue. The other two peptidases used the tripeptide LEU-GLY-GLY as a substrate, but the activity of the PEP2.1 locus was restricted to muscle and liver samples of *B. brachydactylus* and *T. rimac* and one specimen of *T. jelskii*, whereas the PEP2.2 locus was exclusively active in the blood samples of all populations (except *T. culeus*). The PEP1 locus is monomorphic in all but one species: *B. macrostomus* possesses a second allele 83 in low frequency. The PEP2.2 locus is monomorphic in all *Telmatobius* populations, but in the two *Batrachophrynus* species a second allele 115 occurs in low frequency. No heterozygotes were found at any of the loci.

6-phosphogluconate dehydrogenase

Following electrophoresis in the tris-citrate system, in all tissues we found activity corresponding to the same allele of a monomorphic locus.

Phosphoglucomutase

Enzyme systems corresponding to two loci were resolved in the tris-malate system. The monomorphic PGM1 locus stained with similar activity in all tissues, whereas the polymorphic PGM2 locus was detectable exclusively in the muscle and liver samples. In *Telmatobius* and *B. brachydactylus* allele 80 dominates, in *B. macrostomus* allele 100. A total of 6 heterozygotes was found: three of the constitution 80/100 (*T. rimac* from Obrojillo), one of 80/113 and two of 100/113 (*T. jelskii* from Palian).

Hemoglobin

In the tris system the distinction of bands corresponding to hemoglobin was best. We found two alleles present in all central Peruvian populations, allele 100 dominating in the *Telmatobius*, allele 120 in the *Batrachophrynus*. Heterozygotes were not detected.

Table I. - Allele frequencies at the polymorphic loci in 7 samples of 5 species of Andean Telmatobiinae (genera *Batrachophrynus* and *Telmatobius*). P %: relative frequency of polymorphic loci; A: average number of alleles per locus; H_e. relative frequency of expected heterozygosity; H_o: relative frequency of observed heterozygosity.

Locus +	Alleles	*B. brachydactylus* N = 22	*B. macrostomus* N = 21	*T. jelskii* (Cuyro.) N = 9	*T. jelskii* (Palian) N = 21	*T. rimac* (Huaytara) N = 15	*T. rimac* (Obrojillo) N = 22	*T. culeus* N = 1
AAT	60	-	-	0.143	0.059	0.938	0.952	no activity detectable
	100	1.000	1.000	0.857	0.941	0.062	0.048	
AK	75	0.052	-	-	-	-	-	no activity detectable
	100	0.789	0.900	0.571	0.824	0.813	0.833	
	120	0.159	0.100	0.429	0.176	0.187	0.167	
EST1	50	0.077	-	-	-	-	-	-
	90	0.154	0.231	-	0.286	0.083	0.125	-
	100	0.769	0.769	1.000	0.714	0.917	0.875	1.000
EST2	90	0.105	0.053	-	-	0.125	0.111	-
	100	0.895	0.947	1.000	1.000	0.875	0.889	1.000
EST3	94	0.111	0.421	0.429	0.063	0.125	0.167	-
	100	0.889	0.579	0.571	0.937	0.875	0.833	1.000
EST4	92	-	0.053	-	0.067	0.125	0.412	-
	100	1.000	0.947	1.000	0.933	0.875	0.589	1.000
GPI	73	0.091	0.050	0.333	0.278	1.000	0.950	-
	100	0.909	0.950	0.667	0.722	-	0.050	1.000
HDH	75	0.545	0.395	0.750	0.850	0.875	0.579	no activity detectable
	100	0.455	0.605	0.250	0.150	0.125	0.421	
IDH	93	0.091	0.050	-	-	-	-	no activity detectable
	100	0.909	0.950	1.000	1.000	1.000	1.000	
IDH	90	0.600	0.400	-	-	-	0.071	no activity detectable
	100	0.300	-	1.000	0.667	1.000	0.929	
	110	-	0.400	-	-	-	-	
	125	0.100	0.200	-	0.333	-	-	
LDH1	33	1.000	1.000	-	-	-	-	-
	100	-	-	1.000	1.000	1.000	1.000	1.000
MDH1	71	0.050	0.067	0.444	0.188	0.688	0.556	-
	100	0.950	0.933	0.556	0.912	0.312	0.444	-
	125	-	-	-	-	-	-	1.000
MDH2	85	0.111	0.167	0.500	0.400	0.563	0.262	-
	100	0.833	0.750	-	0.600	0.437	0.643	1.000
	115	0.056	0.083	0.500	-	-	0.095	-
ME	20	0.167	0.026	-	-	-	0.053	-
	60	0.286	0.474	-	0.063	0.438	0.395	-
	100	0.547	0.500	1.000	0.937	0.562	0.552	1.000
PEP1	83	-	0.125	-	-	-	-	-
	100	1.000	0.875	1.000	1.000	1.000	1.000	1.000
PEP2.2	100	0.714	0.750	1.000	1.000	1.000	1.000	no activity detectable
	115	0.286	0.250	-	-	-	-	
PGM2	80	0.611	0.400	0.857	0.500	1.000	0.750	no activity detectable
	100	0.389	0.600	-	0.333	-	0.250	
	113	-	-	0.143	0.167	-	-	
Hb	100	0.136	0.444	0.889	0.455	0.875	0.563	1.000
	120	0.864	0.556	0.111	0.545	0.125	0.437	-
P %		0.61	0.68	0.41	0.62	0.50	0.64	
A		1.80	1.86	1.41	1.67	1.50	1.73	
H_e		0.30	0.29	0.39	0.30	0.28	0.31	
H_o		0.04	0.01	0.00	0.03	0.01	0.04	

Table II - Matrix of genetic distances among six samples of four central Peruvian species of Andean Telmatobiinae (genera *Batrachophrynus* and *Telmatobius*).

A. Nei's genetic distance

Species + Population	*B. macrostomus*	*T. jelskii* (Cuyro)	*T. jelskii* (Palian)	*T. rimac* (Huaytara)	*T. rimac* (Obrojillo)
B. brachydactylus	0.0301	0.2237	0.1204	0.3177	0.2613
B. macrostomus	-	0.2304	0.1417	0.3464	0.2874
T. jelskii (Cuyro)		-	0.0661	0.1055	0.1296
T. jelskii (Palian)			-	0.1387	0.1232
T. rimac (Huaytara)				-	0.0245

B. Cavalli-Sforza's chord distance

Species + Population	*B. macrostomus*	*T. jelskii* (Cuyro)	*T. jelskii* (Palian)	*T. rimac* (Huaytara)	*T. rimac* (Obrojillo)
B. brachydactylus	0.1211	0.5993	0.3667	0.6996	0.5434
B. macrostomus	-	0.6621	0.4227	0.7768	0.6131
T. jelskii (Cuyro.)		-	0.2349	0.3092	0.3408
T. jelskii (Palian)			-	0.3123	0.2641
T. rimac (Huaytara)				-	0.803

C. Reynolds's genetic distance

Species + Population	*B. macrostomus*	*T. jelskii* (Cuyro.)	*T. jelskii* (Palian)	*T. rimac* (Huaytara)	*T. rimac* (Obrojillo)
B. brachydactylus	0.0895	0.4575	0.3009	0.5494	0.4481
B. macrostomus	-	0.4443	0.3174	0.5458	0.4501
T. jelskii (Cuyro)		-	0.2238	0.3512	0.3365
T. jelskii (Palian)			-	0.3840	0.3035
T. rimac (Huaytara)				-	0.1004

Table III - Matrix of Nei's genetic distances among four north Peruvian *Telmatobius* species, calculated from the data published in WIENS (1993).

Species	*Telmatobius latirostris*	*Telmatobius necopinus*	*Telmatobius truebae*
Telmatobius brevipes	0.9246	0.8504	0.8702
Telmatobius latirostris	-	0.4479	0.3411
Telmatobius necopinus		-	0.4973

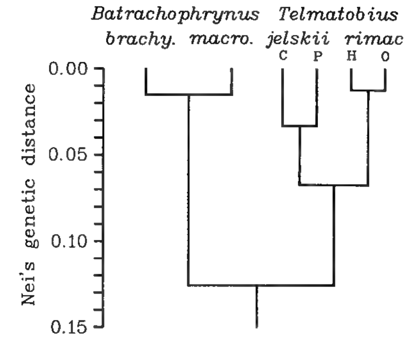


Fig. 1. — UPGMA dendrogram of genetic similarity among six samples of four central Peruvian species of Andean Telmatobiinae, based on the matrix of Nei's genetic distances (Table II A).

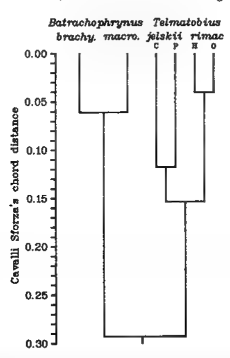


Fig. 2. — UPGMA dendrogram of genetic similarity among six samples of four central Peruvian species of Andean Telmatobiinae, based on the matrix of Cavalli-Sforza's chord distances (Table II B).

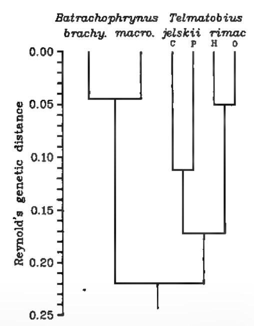


Fig. 3. — UPGMA dendrogram of genetic similarity among six samples of four central Peruvian species of Andean Telmatobiinae, based on the matrix of Reynolds's genetic distances (Table II C).

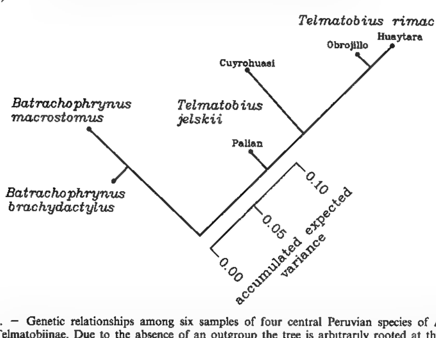


Fig. 4. — Genetic relationships among six samples of four central Peruvian species of Andean Telmatobiinae. Due to the absence of an outgroup the tree is arbitrarily rooted at the mean distance between the most similar populations of *Batrachophrynus* and *Telmatobius*. Maximum likelihood method, based on the allele frequencies (Table I); best tree out of 202 examined in five runs: ln likelihood = 88 3.

Phylogenetic relationships

Allele frequencies (Table I) obtained for the six populations of four central Peruvian telmatobiine species were used for the calculation of three measures of genetic distances (Table II). The reconstruction of the phylogenetic relationships by four commonly used algorithms was based on either allele frequencies or distance matrices (figs. 1-4).

Independent of the algorithm used for the calculation of the genetic distances among the populations sampled, the matrices obtained shared the following features: (1) the genetic differentiation between the two populations of *T. rimac* and between the two *Batrachophrynus* species was low and at the same level; (2) the level of differentiation between the two *T. jelskii* populations was 2-3 times greater than between the *T. rimac* populations, and the *Batrachophrynus* species; (3) the *Telmatobius* species were genetically more similar to each other than any to the *Batrachophrynus* species.

All algorithms based on distance matrices produced identical groupings of populations. Therefore, only the UPGMA-dendrograms are shown as representative examples (figs. 1-3). The main clusters corresponded to the genera *Batrachophrynus* and *Telmatobius* and conspecific populations were placed together. The unrooted maximum likelihood tree based on allele frequencies shows a similar grouping of the populations (fig. 4). However, the *T. jelskii* populations are placed on different branches of the *Telmatobius* lineage.

In addition to the four central Peruvian species, we also included the southern Peruvian *T. culeus* into the analysis. As the data on the allozymes expressed in this species are based on one individual, a quantitative approach to the phylogenetic relationships is not possible yet. Nevertheless, a qualitative reconstruction based on the presence or absence of alleles was attempted (fig. 5). Four monomorphic loci and several common alleles at the polymorphic loci justify the common root of the dendrogram. The distinction between *Batrachophrynus* and *Telmatobius* is based on fixed alleles at the LDH1 locus. *T. culeus* differs from *T. jelskii* and *T. rimac* by fixed alleles at the MDH1 locus. In *B. brachydactylus* and *B. macrostomus*, and in *T. jelskii* and *T. rimac*, respectively, we were unable to find a locus with different fixed alleles. Species distinction is based on the presence and absence, respectively, of rare alleles at the EST2-locus and the PEP1-locus.

Discussion

The radiation of a late tertiary stock of telmatobiine frogs into the recently uplifted cordilleran environments led to a differentiation of about 30 presently known species (Cei, 1986; Duellman, 1993; Frost, 1985; Wiens, 1993). Allopatric populations which inhabit numerous interandean valleys and streams of the Pacific or Atlantic hydrographic systems give an idea of the mechanisms of speciation at work. The geomorphologically complicated Late Pleistocene landscape favoured the formation of disjunct populations (Cei, 1986). Progressive genetic changes in isolated gene pools probably promoted allopatric speciation in the Andean Telmatobiinae. The tendency for homozygosity at almost all loci in the Andean populations already studied is a strong indication of small

population size and interrupted gene flow between populations. Large genetic distances between populations assigned to *T. jelskii* and inhabiting different river systems demonstrate that allopatric speciation is still the norm at the present time.

Early attempts to analyze relationships between the Andean Telmatobiinae (VELLARD, 1951, 1953, 1955) mainly reflect groupings assigned by biogeographical convenience to a poor data base. LYNCH (1978) was the first to use a cladistic approach on a set of morphological and osteological data. He placed the Andean genera *Batrachophrynus* and *Telmatobius*, along with additional seven genera (*Alsodes*, *Atelognathus*, *Eupsophus*, *Hylorina*, *Insuetophrynus*, *Limnomedusa* and *Somuncuria*), into the tribe Telmatobiini Fitzinger, 1843. All cladograms (based on differing numbers of OTUs) emphasized that the common ancestor of the lineage leading to the two *Batrachophrynus* species separated at a very early stage from the lineage leading to the present *Telmatobius* species. Nevertheless, DUELLMAN's (1979: 424) statement: "the systematic relationships of the species of *Telmatobius* presently are too poorly known to assess fully the historical biogeography of the group" remains valid in spite of increased knowledge on the Andean Telmatobiinae accumulated since VELLARD's pioneer work.

Our attempt to reconstruct phylogenetic relationships of the central Peruvian Telmatobiinae is based on allozymes. The results correspond to those on northern Peruvian *Telmatobius* (21 individuals, 4 species, 19 loci) in terms of low heterozygosity at most loci (WIENS, 1993). However, the Nei's distances among the species which we studied are generally lower than those among four northern Peruvian *Telmatobius* species (Table III, calculated from Table 5 in WIENS, 1993). This discrepancy is probably due to differences in method (e. g., number and kind of loci scored, number of individuals, buffer systems, resolution of gels). All dendrograms and trees derived from processing allele frequencies and distance matrices indicate the same sequence of speciation events among the four species of *Batrachophrynus* and *Telmatobius* we examined.

Allozymes clearly support monophyly in *Batrachophrynus*. The gene pools of *B. brachydactylus* and *B. macrostomus* were surprisingly similar and the genetic distance of 0.03 between the two species is the lowest interspecific Nei's distance ever reported for Amphibia (usually 0.1 to 3.0; AVISE & AQUADRO, 1982). LAURENT's (1983) hypothesis of an independent derivation from the *Telmatobius* stock is not supported. This hypothesis apparently resulted from a misinterpretation of convergent morphological traits in the stream-inhabiting *B. brachydactylus* and *Telmatobius* species.

We were unable to detect any fixed genetic difference between the two *Batrachophrynus* species and even allele frequencies are very similar. Taken alone, allozymes would suggest that *B. brachydactylus* and *B. macrostomus* are conspecific. On the other hand, these taxa are morphologically well defined, extremely different in size, and they live in different habitats (PETERS, 1873; SINSCH, 1990; SINSCH et al., 1995). Intermediate individuals between the *brachydactylus* phenotype and the *macrostomus* phenotype are not known. Personal field observations in the area of Lake Junín, where both taxa occur sympatrically, did not yield any evidence of interbreeding. In conclusion, despite the low differentiation of the *Batrachophrynus* gene pool for the allozymes we studied (comparable to studies on bird allozymes), we do not doubt the specific status of both taxa which we consider sister species.

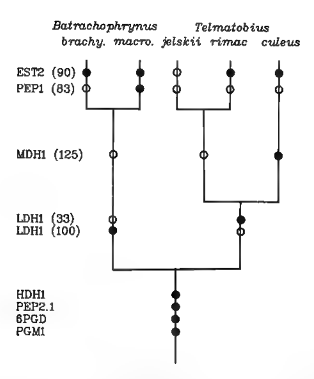


Fig. 5. — Proposal for the phylogenetic relationships among five species of Andean Telmatobiinae, based on the presence (dot) and absence (circle), respectively, of alleles at different allozyme loci.

The cluster formed by *Telmatobius* samples agrees with the morphometric assignment of populations to the species *T. jelskii* and *T. rimac*. The genetic distance between the conspecific samples correlates with the corresponding geographical distance of localities. Finally, the low genetic distance between *T. jelskii* and *T. rimac* as well as the absence of diagnostic fixed alleles indicates a close phylogenetic relationship.

The evaluation of fixed alleles and of presence/absence of rare alleles supports the same tree structure as the quantitative treatment of allele frequencies. Moreover, it allows for a proposal on the relationships of *T. culeus* with the central Peruvian taxa (fig. 5). Yet, the large geographical distance between the current distribution ranges of *T. jelskii* and *T. rimac* on one hand, and *T. culeus* on the other, indicates a long period of independent evolution. Nevertheless, the fixed allele at the LDH1 locus clearly suggests that *T. culeus* is a member of the same *Telmatobius* lineage as are the central Peruvian species. This shared character state distinguishes *Telmatobius* from *Batrachophrynus* and favours the hypothesis of an early separation put forward by Lynch (1978). The geographically distant *T. culeus* genetically and morphometrically resembles the *Batrachophrynus* species more than the neighboring central Peruvian *Telmatobius* species do. This may indicate that *Batrachophrynus* species represent the remnants of an early invasion into the central Peruvian Andes, whereas the *Telmatobius* species reached this region during a second, later invasion. The limited present range of distribution of *Batrachophrynus*, and the suspicious

absence of streams occupied by both *B. brachydactylus* and a stream-inhabiting *Telmatobius* species, suggest that *Telmatobius* is competitively superior to *Batrachophrynus*. Future field work in the contact zone between *B. brachydactylus* and *T. jelskii* in the streams of the Junín area should reveal whether the two can coexist in the same stream.

Our study supports the taxonomic distinction of the central Peruvian Telmatobiinae into two genera *Batrachophrynus* and *Telmatobius* (PETERS, 1873). The geographical distribution of *Batrachophrynus* and *Telmatobius* species indicates the result of competition between early and late invaders of this region rather than phylogenetic proximity. However, the genetic distance between the members of these genera is low. Data from additional *Telmatobius* species and from an appropriate outgroup (*Alsodes*, considered as sister taxon of *Telmatobius*) are needed for a final decision on the relationships between *Batrachophrynus* and *Telmatobius*.

RESUMEN

Se revisan tres hipótesis sobre el origen filogenético de los Telmatobiinae del Perú central: (1) el antepasado común de las dos especies de *Batrachophrynus* se separó del stock de *Telmatobius* antes de su diferenciación en las especies recientes; (2) *B. macrostomus* se separó primero, *B. brachydactylus* más tarde del stock de *Telmatobius*; (3) la separación del antepasado común de *Batrachophrynus* del stock de *Telmatobius* ocurrió despues de su diferenciación. Alocimas demuestran claramente que *B. brachydactylus* y *B. macrostomus* son familiares muy cercanos, y que *T. culeus* del sur del Perú es relacionado más cercano con *T. jelskii* y *T. rimac* del Perú central que con las especies de *Batrachophrynus*. En conclusión, todas las pruebas disponibles apoyan la primera hipótesis.

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Advertisement, aggressive, and possible seismic signals of the frog *Leptodactylus syphax* (Amphibia, Leptodactylidae)

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Advertisement calls from three geographically isolated populations of *Leptodactylus syphax* are remarkably similar. Aggressive calls of *L. syphax* sound to the human ear very different from the advertisement calls, although the basic structural components are similar. A male *L. syphax* responded with aggressive calls to a playback of his own advertisement call. The same male responded to playbacks of his aggressive calls with increased rate of aggressive calls and foot pounding behavior. The foot pounding produced audible clicks and, by its nature, seismic signals. This is the second known instance of *Leptodactylus* species producing seismic signals, each produced differently, however. It is not known whether *L. syphax* interprets the seismic signals. Seismic signalling in frogs may be much more common than currently believed.

Introduction

Leptodactylus syphax Bokermann, 1969 is restricted to rocky granitic outcroppings and is known from a few disjunct, widely separated, localities in Brazil (fig. 1). The first author recently recorded calls from three of these disjunct populations. We analyze the advertisement calls of the frogs from these three populations to determine whether there is any significant variation among them. At one locality, the first author was fortunate to observe and record *Leptodactylus syphax* aggressive calls and foot-pounding behavior. The foot-pounding may involve seismic communication, previously reported for the first time in frogs by Lewis & Narins (1985). We describe and comment on all of these calls and behaviors.



Fig. 1. – Known distribution of *Leptodactylus syphax* in South America. Triangles: sites from which recordings are analysed in this paper (westernmost triangle: Barra do Bugres, Mato Grosso State; northernmost triangle: São Raimundo Nonato, Piauí State; southernmost triangle: Alpinópolis, Minas Gerais State). Square: site from previously published recording by W. C. A. Bokermann (Chapada dos Guimarães, Mato Grosso State). Dots: other known localities (note that southernmost dot, the locality of Serra do Espinhaço, Minas Gerais State, was incorrectly placed in northeastern Brazil in Heyer, 1979, fig. 21).

METHODS AND MATERIALS

Recordings were made using a Uher Report 4000 reel-to-reel tape recorder. The recording information is:

(1) Tape ASN/AJC (Archivo Sonoro Neotropical/Adão J. CARDOSO) 13, cut 6, Brazil, Minas Gerais State, Alpinópolis, Fazenda Salto; no voucher specimen; recorded by A. J. CARDOSO; 11 October 1981; 21.00 hours; 22°C air temperature. Ten advertisement calls are analyzed from this individual.

(2) Tape ASN/AJC 84, cut 2, Brazil, Mato Grosso State, Barra do Bugres, Reserva Biológica Serra das Araras; no voucher specimen; recorded by A. J. CARDOSO; 19 November 1988; 20.30 hours; 26°C air temperature. Sixteen advertisement calls are analyzed from this individual.

(3) Tape ASN/AJC 101, cut 2, Brazil, Piauí State, São Raimundo Nonato, Parna, Serra da Capivara, localidade Caldeirão; voucher specimen ZUEC 8829 (Universidade Estadual de Campinas); recorded by A. J. CARDOSO; 4 March 1990; 20.00 hours; 27.5°C air temperature. Ten advertisement calls, 9 aggressive calls, and 1 foot pounding are analyzed from this individual.

The recordings were analyzed with "Canary" software from the Cornell Laboratory of Ornithology on a Macintosh IIci computer. The sampling rate used to convert the analogue signals to digital format was 22,254.5 Hz with 8-bit precision. Filter bandwidths of 353 Hz and frame lengths of 256 points were used for both audiospectrogram and spectrogram analyses.

Call terminology follows that defined in HEYER et al. (1990).

RESULTS

ALPINÓPOLIS DATA

Three individuals were calling at the recording site. The calling males were separated from each other by a distance greater than 100 m, far from water, in an area characterized by large rocks, among which crevices were abundant. The individual recorded was calling near one of these crevices, into which it fled after being approached to within about 10 m. Only the advertisement call was recorded; no playback was presented to the frog.

The advertisement call (fig. 2 A), is given at an average rate of 0.8 per second. Call duration ranges from 59 to 64 ms. The call is frequency modulated with a rapid rise time; the broadcast frequency range sweeps from 390 to 2110 Hz with maximum broadcast intensity between 1310 and 1330 Hz. The call is strongly partially pulsed, typically with 3 almost completely defined pulses. Harmonics are present (not particularly visible on fig. 2 A, but spectrogram analyses of calls [not shown] indicate their presence).

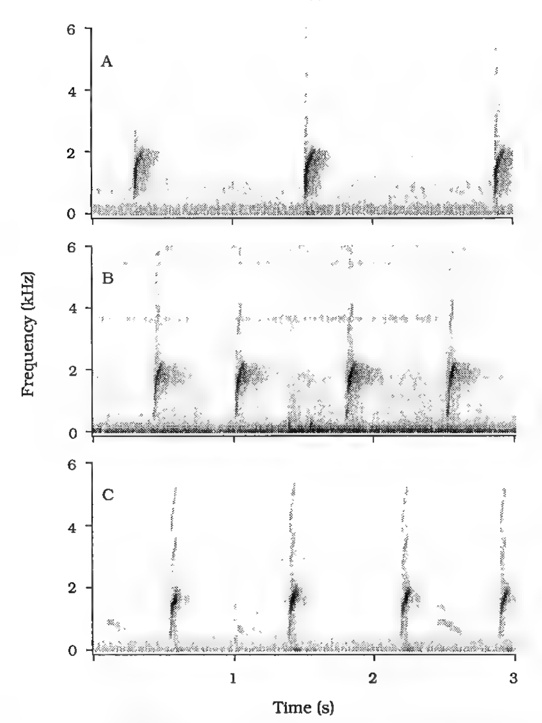


Fig. 2. — Audiospectrograms of advertisement calls of *Leptodactylus syphax*. Upper figure (A) recorded from Alpinópolis; middle figure (B) recorded from Barra do Bugres; lower figure (C) recorded from São Raimundo do Nonato.

BARRA DO BUGRES DATA

A single individual was calling from this locality, which was characterized by large sheets of rock and no water systems obvious in the area. Advertisement calls only were recorded. The individual stopped calling when approached within about 20 m and did not respond to playback of its call when broadcast from near the calling site.

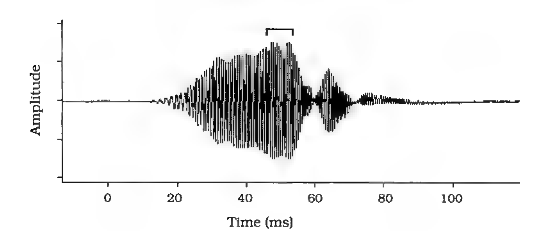
The advertisement call (fig. 2 B) is given at an average rate of 1.5 per second. Call duration ranges from 53 to 60 ms. The call is frequency modulated with a rapid rise time; the broadcast frequency range sweeps from 380 to 2300 Hz with maximum broadcast intensity between 1800 and 1850 Hz. The call is partially pulsed, with 3 to 5 weakly defined pulses (the recording has a low frequency component making precise determination of the number of pulses difficult). Harmonics are present (energy analyses of calls [not shown] indicate their presence).

SÃO RAIMUNDO NONATO DATA

Two individuals were calling at the site, about 200 m from each other. The individual recorded was calling from an extensive horizontal rock fissure. The opening of the crevice was about 30 cm high and 5 m long. The crevice extended about 4 m into the rock wall, on the face of a waterfall, which at the time had little running water.

Initially the advertisement call was recorded without the monitor on, such that the frog did not hear its own voice. At this time, the calling frog was about 4 m from the microphone. After the initial recording was made, the monitor button was engaged and the frog began to hear its own voice from the tape recorder speaker. Immediately after hearing its own voice, the individual started to emit aggressive calls intermixed with advertisement calls and jumped to within about 2 m of the tape recorder. The emissions were given at a very variable rate with considerable irregularity in the bursts of call types. After a while of recording under these conditions, a section of tape with a series of aggressive calls was played back to the frog. On hearing the playback of these aggressive calls, the frog increased its rate of aggressive calls and beat its forelimbs on the ground, thereby causing the foot pounding sound. The tape recorder was then stopped, recording was begun anew with the monitor engaged, such that the frog could hear its own sounds from the tape recorder speaker, including the foot pounding sounds. Soon thereafter, the frog jumped to the side of the tape recorder, emitted various sounds, and abruptly stopped calling. The frog was then collected to serve as a voucher for the recordings.

The advertisement call (figs. 2 C, 3), is given at an average rate of 1.2 per second. Call duration ranges from 56 to 64 ms. The call is frequency modulated with a rapid rise time; the broadcast frequency sweeps from 390 to 2060 Hz with maximum broadcast intensity between 1640 and 1680 Hz. The call is either composed of two extremely well-defined pulses (almost notes), the first with about 3 weakly defined partial pulses, or composed of about 4 weakly defined partial pulses. Harmonics are present.



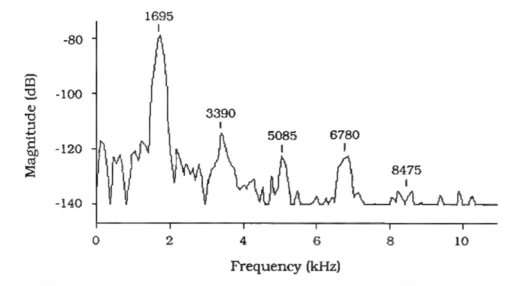


Fig. 3 — Wave form and energy analysis of advertisement call of *Leptodactylus syphax* recorded from São Raimundo do Nonato. Upper figure shows wave form (the pulse above the 80 milliseconds label is interpreted as microphone ringing, not a part of the call); bracket above wave form indicates portion of call used for spectrogram analysis of lower figure.

The aggressive call (figs. 4, 5) is given at an average rate of 0.7 per second. Call duration ranges from 162 to 206 ms. The call is frequency modulated in a complex fashion. There is an initial low intensity fast rise in frequency, the fundamental rising from 220-480 Hz to 920-1010 Hz, followed by a falling frequency, steeper initially, the fundamental from about 920-1010 Hz falling to 310-520 Hz. The call is partially pulsed with about 3 weakly defined pulses. There are at least 3 well-defined harmonics which have about as much broadcast energy as the fundamental.

There is no apparent transition in call when a male switches from advertisement to aggressive calls (and vice versa); the male utters either one kind or the other (fig. 5).

The pounding of the front foot results in a faint, but audible click (fig. 4), that has most energy at about 1800 Hz. Foot pounding, by its nature, produces seismic signals as well.

DISCUSSION

The calls from the three isolated populations of *Leptodactylus syphax* studied are remarkably similar, differing only in details that might be expected to occur among individuals from a single population (see GERHARDT, 1988, for a general discussion and RYAN, 1980, for a specific example analyzing fundamental frequency). The calls reported here are also similar to the call from Chapada dos Guimarães, Mato Grosso State, recorded by Werner C. A. BOKERMANN, previously reported (HEYER, 1979), with one exception. The previously analyzed recording from Chapada dos Guimarães gave very little indication of harmonic structure. However, harmonic structure is evident in the wave forms of the calls analyzed herein (e. g., fig. 3, above), and the spectrogram analysis indicates the presence of at least 3 harmonics in addition to the fundamental frequency (fig 3, below). These differences in harmonic expression may be due to differences of recording and analytic equipment rather than actual call differences.

The modest differences in advertisement calls among the geographic samples analyzed are somewhat surprising. We do not know whether these similarities may be due to recent isolation of the presently disjunct populations of *L. syphax* or due to selection for stabilization of the advertisement call among all populations.

The advertisement and aggressive calls are very different sounding (and appearing when analyzed) calls. They sound as though they were calls of two different species of frogs. The advertisement and aggressive calls differ in duration and mode of frequency modulation. The calls do share the characteristics of being frequency modulated, having overlapping broadcast frequencies, and having harmonic structure. These similarities suggest that the same physical structural complex is involved in producing both calls and the differences are produced by a combination of behavioral controls regulating the duration of the call and manipulating tension of the laryngeal musculature which causes changes in the tension of the vocal cords resulting in differences of the physical structure of the emissions. These behavioral changes are not trivial, however. LEWIS & NARINS (1985) reported similar results for *Leptodactylus albilabris*. While the advertisement call of

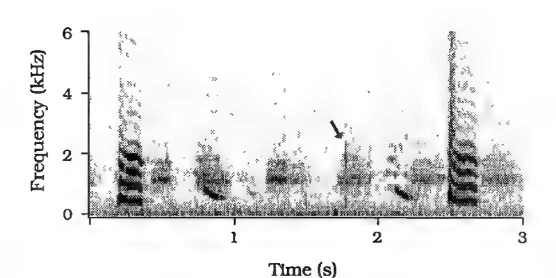


Fig. 4 – Audiospectrogram of two aggressive calls and foot-pounding of *Leptodactylus syphax* recorded from São Raimundo Nonato. The arrow indicates the foot-pounding sound.

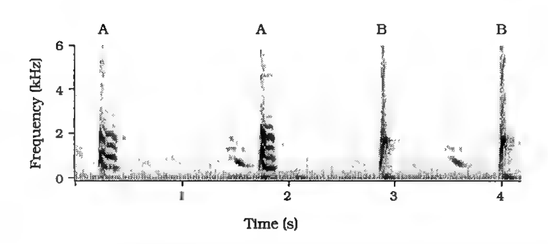


Fig. 5. – Audiospectrogram of continuous recording of a male *Leptodactylus syphax* from São Raimundo Nonato, uttering aggressive calls (A) followed immediately by advertisement calls (B) with no intermediate call structure between the two call types.

L. albilabris is short and rises from 1000 to 2300 Hz, the male-male interaction call is longer and descends from 2300 to 1000 Hz. Perhaps this pattern of frequency modulation reversal and time differences in advertisement and aggressive calls is common to all *Leptodactylus* species with rising whistle-like advertisement calls.

LEWIS & NARINS (1985) and NARINS (1990) reported that *Leptodactylus albilabris* produces and is capable of receiving and interpreting seismic signals. LEWIS & NARINS (1985) speculated that the different arrival times of the simultaneously produced seismic and airborne waves could provide a temporal clue to the distance from the source and could be used to help males establish and maintain territories. NARINS speculated that *L. albilabris* might be able to integrate the seismic and air-borne advertisement calls "to better communicate when high-level background noise obscures the acoustic channel" (NARINS, 1990: 273). This could also pertain to *L. syphax*, as the habitats they call from have noisy waterfalls during rainy periods.

The mechanism for producing seismic signals in *L. albilabris* was reported to be the rapidly expanding vocal sac contacting the ground. *Leptodactylus syphax* produce seismic signals by beating their forefeet on the ground. In contrast to the seismic signals of *L. albilabris*, which are not audible to the human ear, the foot pounding of *L. syphax* is weakly audible to the human ear, and is certainly within the frequency range of the advertisement call of *L. syphax*. We assume the audible nature of the foot pounding of *L. syphax* is possibly due to the presence of horny spines on the inner thumb of the male. In contrast to *L. albilabris*, the seismic signals made by *L. syphax* are not produced simultaneously with advertisement or aggressive calls. We do not know whether *L. syphax* is processing the air-borne click portion of the foot-pounding, the seismic signals, neither, or both. However, we report here the second known instance in frogs producing seismic signals, both within the genus *Leptodactylus*, but by very different methods. Obviously, this foot-pounding behavior of *L. syphax* merits further study as well as detailed study of other frogs to determine whether seismic signalling is much more common than currently believed.

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Captive maintenance of adults and juveniles of the genus *Triturus* during the terrestrial phase

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Two methods for maintaining newts during the terrestrial phase, such that regular recapture could be effected, were compared. The first method, which has been used previously by other workers for plethodontids, led to failure to thrive and absence of breeding condition during the following spring. The second method, which sought to mimic the natural terrestrial habitat of species of the genus *Triturus*, enabled efts to reach sexual maturity in one year and adults to subsequently come into breeding condition.

INTRODUCTION

The development of a method to maintain newts in the terrestrial phase, whereby individuals can easily be recaptured, is important for several reasons. Little is known about growth of juveniles or adults during this phase in the wild, mainly due to the difficulty of locating individuals (GRIFFITHS, 1984). Enhanced feeding of efts may lead to sexual maturity within one year which may assist reproductive studies on captive populations (BAKER, 1988; ELEBERT, 1991). However, raising juveniles to sexual maturity in one year may not be desirable for programmes where the newts are to be re-introduced into the wild, as it may impose unnatural selection pressures.

Newts, captured aquatically, can rapidly lose breeding condition. Males often react to the stress of capture by rapid regression of their crests or tail filaments and both sexes may cease courtship behaviour. VERRELL (1982) found that these reactions to stress in the male could be overcome in *Notophthalmus viridescens* by enhanced feeding but I found this regime to be unsuccessful for both *Triturus montandoni* and *Triturus helveticus* (unpublished data). Maintenance of adults during the terrestrial phase, enabling them to come into breeding condition the following spring, may facilitate studies of courtship behaviour in the laboratory.

Workers in North America have maintained plethodontids terrestrially, which enabled them to observe courtship behaviour for eight months of the year (SEVER & HOUCK, 1985). This method has also been successfully used to rear *Notophthalmus* efts (VERRELL, 1983).

Below, two methods are described, which were investigated for maintaining *Triturus* species during the terrestrial phase; the first method is based on that used for plethodontids and the second method is an attempt to mimic the natural terrestrial habitat of *Triturus* species.

Methods and results

First method

Adult *Triturus helveticus* were collected from a pond in Bedfordshire, England, and allowed to mate in tanks (30 cm × 60 cm × 38 cm high, size 1) at 14°C and on a natural photoperiod The resulting larvae were fed on *Daphnia* and *Tubifex*. At metamorphosis, the efts were transferred to small, round glass dishes (10 cm diameter × 4 cm high), lined with moist paper towel, containing crumpled paper for them to hide under and covered with "parafilm" to maintain the humidity and to prevent their escape. Each week the moist towel was renewed. The efts were kept at a density of six to a dish and maintained at 10-12°C with a 12L:12D photoperiod and fed on fruit flies (*Drosophila*) ad libitum.

After initially emerging from the water onto rocks, the adult newts were observed to re-enter the water several times before their skins reverted to the terrestrial velvety condition. They were then transferred to transparent boxes (17 cm × 31 cm × 9 cm high), at a density of four to a box and maintained under the same conditions as the efts.

The efts failed to thrive; they grew fast initially but then remained at a small size and subsequently died. None reached sexual maturity. The adults tended to lose weight during the terrestrial phase, appearing very thin and dark skinned; none came into breeding condition the following spring

Second method

Triturus alpestris adults were collected in France in 1989, in breeding condition. They were housed in aquaria (size 1) and maintained at 12°C on an artificial photoperiod that replicated the natural photoperiod (condition A). The resulting larvae were fed on *Daphnia* and *Tubifex*. Ten larvae metamorphosed during late summer.

Triturus montandoni adults were collected in Poland in 1990, in breeding condition. However, when they reached the laboratory, the males' tail filaments had regressed and they failed to court. The females, which had been ovipositing prior to capture, failed to deposit any more eggs. Within a few weeks the adults left the water, via rocks emerging from it, and were transferred to a terrarium.

The efts and adults were kept in terraria consisting of transparent plastic tanks (21 cm × 40 cm × 25 cm high) covered with a plastic lid containing mesh over the air holes. A layer of earth, 5 cm deep, was put in the bottom of the tank. Dry leaf litter, which contained small invertebrates (wood lice, ants, beetles, etc.), was placed on top of the earth until the tank was half-full, followed by several large stones and some pieces of bark for the newts to hide under. The terraria were maintained so that the soil base was always moist and the leaf litter dry. Each terrarium contained up to ten efts or six adults, which were fed on *Drosophila* (flies and maggots) and white worms (*Enchytraeus albidus*) ad libitum, and maintained at 19-23°C with a natural photoperiod. One corner of the terrarium was used to maintain the white worms, which were replenished regularly.

In December, the terraria were transferred to condition A, to simulate winter, and fed as above. In February, when *Triturus vulgaris* were migrating to the ponds locally, the adult *T. montandoni* were transferred to aquaria (size 1), filled to a depth of 15 cm, and kept in an unheated shed with a natural photoperiod. The *T. alpestris* efts had also thrived and their skins now appeared damp, so they were transferred to tanks identical to the above. The newts were placed on bricks above the water level.

In the terraria, the newts were often found clustered together under a piece of bark and the efts were found inside the curled leaves. The adults were also found buried in the soil, however they could still be seen foraging during the late afternoon and evening.

Within a few days of transfer, the adult *T. montandoni* became aquatic. Subsequently, five of the six came into breeding condition, courted and reproduced. Six juvenile *T. alpestris* became aquatic during the first day. They also came into breeding condition but, as all the juveniles were female, no courtship was observed.

DISCUSSION

Looking at the results described above, it appears that the regime used in North America so successfully for plethodontids is inappropriate for *Triturus* spp. In the first method, although the absolute humidity may change as the week progresses due to the moist towel drying out, the humidity is uniform throughout the box. Little is known about the humidity preferences of terrestrial newts; therefore, the humidity achieved in the box may be unsuitable. The diet in this method is also very uniform. SMITH (1951) described the terrestrial diet of newts as consisting of worms, slugs, snails and insects; therefore, it is possible that the newts are being deprived of essential nutrients when fed solely on a single species of insect.

The second method was equally successful for maintaining both efts and adults. The terrarium was set up so that a humidity gradient existed within it and the newts appeared able to find an appropriate microhabitat. The diet was more varied, the newts were fed on *Drosophila* (flies and maggots), white worms and any invertebrates in the soil and leaf litter. This diet may be closer to that found in the wild and is therefore more likely to supply all the nutrients necessary for spermatogenesis and somatic growth. This method also gave the newts a period of "summer" terrestrial conditions which has been shown to be a requirement for complete spermatogenesis to occur (SAEZ et al., 1992). The lack of this "summer" period in the first method may have contributed to the decline of the newts and may indicate that efts also require these higher temperatures for development.

Development of a method that facilitates recapture in the terrestrial phase may allow investigation of differential feeding and growth rates during this phase. Age accounts for only a small proportion of the total variance in adult body size (HALLIDAY & VERRELL, 1988) and therefore a study of juvenile growth during this phase may help to elucidate further the variation in subsequent adult body size. BAKER (1992) has shown that crest height is related to body condition in *Triturus cristatus*. The above method will enable a

study to be undertaken to investigate the relationship between growth and body condition in the terrestrial phase to the development of secondary sexual characteristics and subsequent reproductive success in the aquatic phase.

Résumé

Deux méthodes pour le maintien en captivité de tritons pendant leur phase terrestre sont décrites et comparées. La première méthode, qui a été déjà utilisée avec succès avec des Pléthodontidés, a abouti à un échec en ce qui concerne la croissance des animaux et leur aptitude à se reproduire au printemps suivant. La deuxième méthode, qui cherche à reproduire l'habitat terrestre naturel des espèces du genre *Triturus*, a permis aux jeunes tritons d'atteindre la maturité sexuelle en un an et aux adultes de retrouver leur condition reproductive au printemps.

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